iGEM 2016: Team Pittsburgh

Week 14 Lab Notebook

Tuesday, August 23

Sequences for PT3-RBS returned (Maya)

All three are confirmed sequences

Linearize Terminator (Maya)

5.49 uL Uncut Term
10.51 uL H2O
1 uL SpeI
1 uL PstI
2 uL Buffer

Annealing of PT7-Toehold (G)

Phosphorylation

5 uL Oligo
2 uL 10x T4 Buffer
1 uL PNK
12 uL H2O

Incubate (37 for 60min, 65 for 20min)

Annealing

5 uL FWD
5 ul Reverse
5 ul 10x Annealing Buffer
35 ul H2O

PCR (95 degree for 3 minutes, then -1 degree every cycle for 60 cycles, at 25 degree for 5 minutes then end)

Ligation of PT3-RBS-T3 PT7-RBS-T3 to terminator (Maya)

1 uL T4 Ligase
2 uL T4 Ligase Buffer
PT3-RBS-T3

Control:	1 ug Vector
	0 ug Insert
	--- ul H2O
3:1	1 ug Vector
	3.857 ug Insert
	--- ul H2O
7:1	1 ug Vector
	9.00 ug Insert
	--- ul H2O

PT7-RBS-T3

Control:	1 ug Vector
	0 ug Insert
	--- ul H2O

3:1	1 ug Vector
	3.857 ug Insert
	--- ul H2O
7:1	1 ug Vector
	9.00 ug Insert
	--- ul H2O

Transform with Cheryl's cells (Maya)
Transform PT7-T3 and Term (Maya)

Wednesday, August 24

Lab meeting
Make lead buffer (Claire and Praneeth)
Each make 30 mL of buffer
50 mM HEPES = 0.357 g
50 mM NaCl = 0.088 g
5 mM $MgCl_2$ = 0.15 mL 1 M solution
pH = 7.26
Cleavage reaction (Praneeth)
[Praneeth's or Claire's buffer] x [37 or RT] x [denatured x original]
5 nM hairpin
2 uM lead
Lead buffer
50 uL reaction
William and Mary constructs in S30 extract (Claire)

DNA	2 ug		
Amino Acids Mix without Methionine	5 uL		80 uL
Premix without Amino Acids	20 uL	x 16 rxn =	320 uL
S30 Extract for Circular DNA	15 uL		240 uL
Water to volume	50 uL		

Sample	DNA volume (uL)	Water volume (uL)
A1	7.72	2.28
A2	10.25	0
B1	6.73	3.27
B2	5.83	4.17
C1	6.58	3.42
C2	6.62	3.38
D1	7.05	2.96

D2	7.83	2.17
E1	7.61	2.39
E2	7.25	2.75
F1	8.74	1.26
F2	7.52	2.48
G1	7.78	2.22
G2	8.79	1.21
H1	7.68	2.32
H2	7.62	2.38
Negative control	0	10

Procedure:

*Keep all tubes on ice

1. Add appropriate amount of DNA into 1.5 mL Eppendorf tubes
2. Combine AA w/o Met, premix, and extract; spin down
3. Add 40 uL of extract into each tube with DNA
4. Add appropriate amount of water to each tube; spin down
5. Aliquot each tube into 3 tubes of 16.66 uL each
6. Incubate at 37 degC for 1 hour
7. Quench on ice for a few minutes

After incubation, add 15 uL of each sample into black 384-well plate

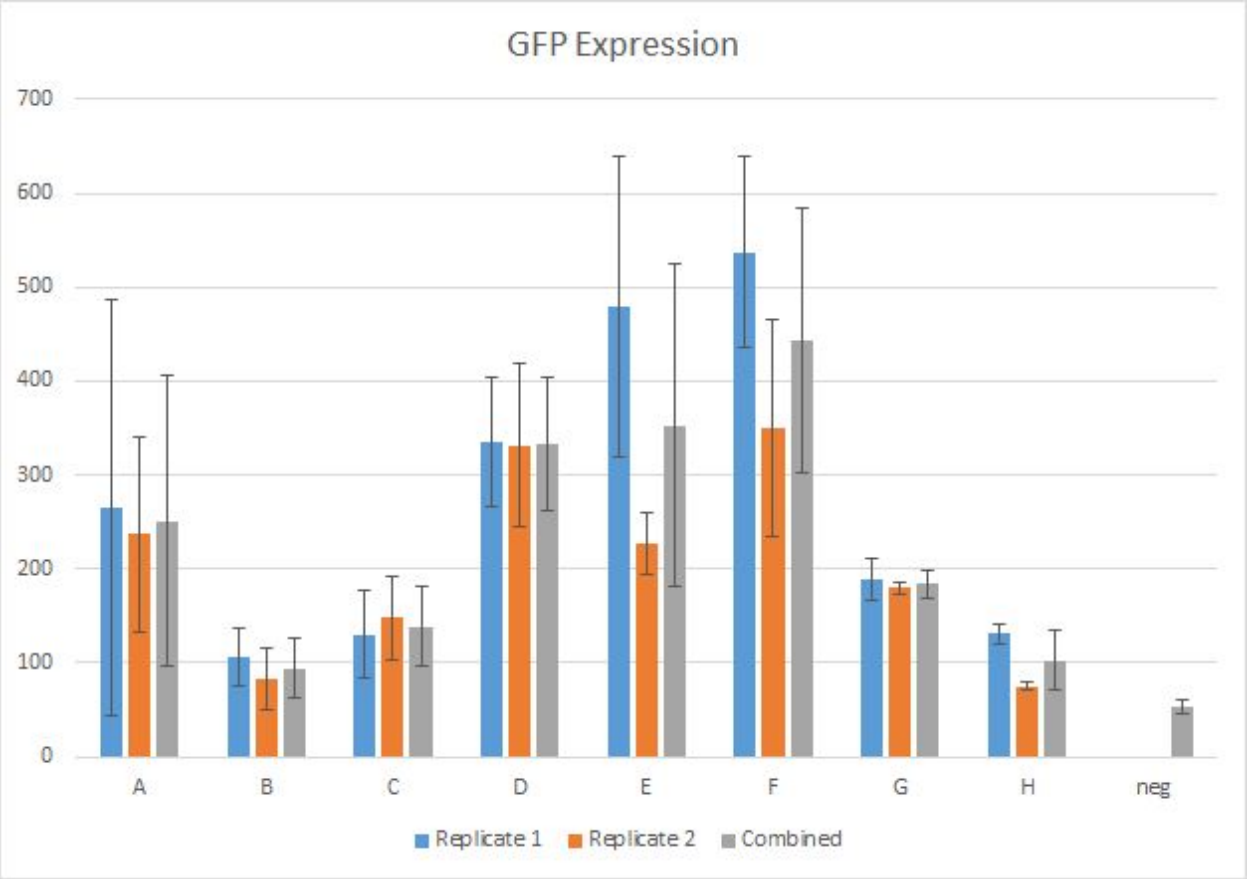
Some samples had bubbles; others were short of 15 uL

Read at 485 excitation, 515 emission, 495 cutoff

Plate:

	1	2	3	4	5	6	7	8	9	10	11	12
E					neg	neg	neg					
F	A1	A1	A1	A2	A2	A2	B1	B1	B1	B2	B2	B2
G	E1	E1	E1	E2	E2	E2	F1	F1	F1	F2	F2	F2
	13	14	15	16	17	18	19	20	21	22	23	24
F	C1	C1	C1	C2	C2	C2	D1	D1	D1	D2	D2	D2
G	G1	G1	G1	G2	G2	G2	H1	H1	H1	H2	H2	H2

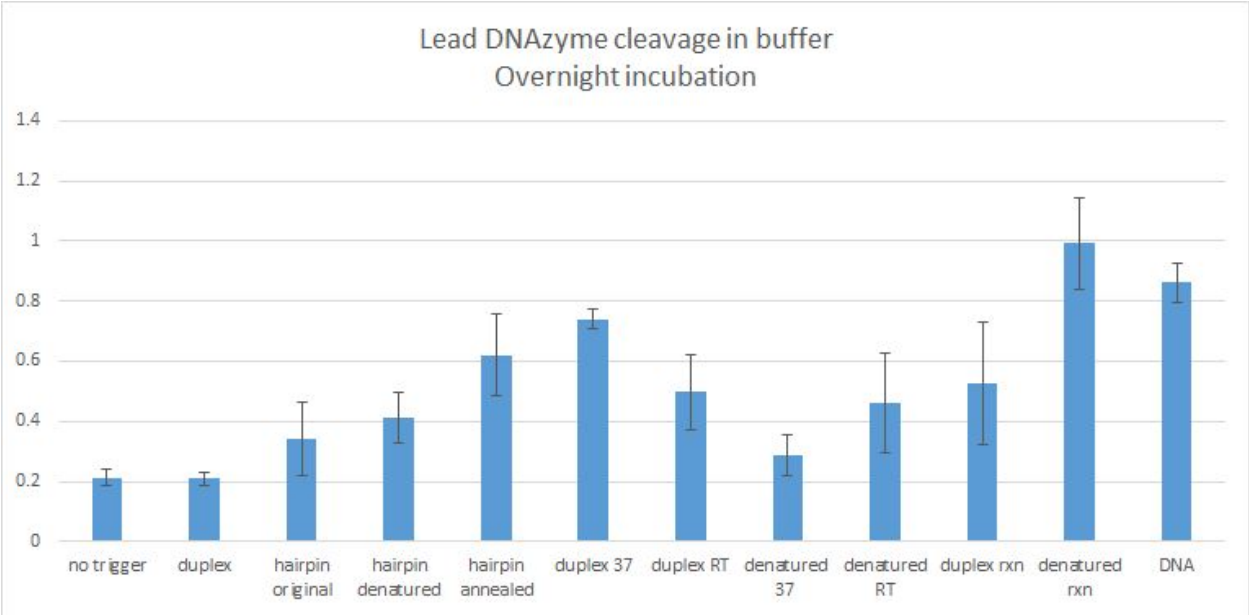
Sample	Replicate											
	1					2					avg	st dev
	1	2	3	avg	st dev	1	2	3	avg	st dev		
A	518.911	116.255	159.943	265.0363	220.9444	351.27	148.001	211.91	237.0603	103.9422	251.0483	155.1868
B	138.98	101.79	77.914	106.228	30.77395	118.23	53.418	75.149	82.26567	32.98688	94.24683	31.40583
C	91.412	182.484	116.734	130.21	47.00776	102.289	189.556	151.515	147.7867	43.7528	138.9983	41.74083
D	296.777	292.849	415.261	334.9623	69.56841	390.287	231.692	371.598	331.1923	86.67501	333.0773	70.32206
E	315.194	487.806	634.543	479.181	159.8491	192.83	257.282	230.684	226.932	32.3894	353.0565	172.4216
F	576.818	421.422	613.221	537.1537	101.8659	219.824	437.542	393.086	350.1507	115.0342	443.6522	141.1908
G	184.221	167.934	212.428	188.1943	22.51154	186.177	178.489	174.624	179.7633	5.880977	183.9788	15.42293
H	125.651	124.512	142.954	131.039	10.3344	75.381	70.383	79.511	75.09167	4.570873	103.0653	31.46599
neg	45.798	59.426	54.426								53.21667	6.894016



Cell-free lead DNAzyme test: read after overnight incubation (Claire)

Condition	1	2	average	st dev
no trigger	0.2307	0.1924	0.21155	0.027082
duplex	0.2255	0.192	0.20875	0.023688
hairpin original	0.4286	0.2556	0.3421	0.122329
hairpin denatured	0.4743	0.3548	0.41455	0.084499
hairpin annealed	0.7189	0.5235	0.6212	0.138169
duplex 37	0.7172	0.7642	0.7407	0.033234
duplex RT	0.5857	0.4086	0.49715	0.125229
denatured 37	0.3355	0.2411	0.2883	0.066751
denatured RT	0.5798	0.3457	0.46275	0.165534

duplex rxn	0.6706	0.383	0.5268	0.203364
denatured rxn	1.0996	0.8834	0.9915	0.152876
DNA	0.9067	0.8159	0.8613	0.064205



Folded hairpin did not completely sequester trigger

Still difference between lead and no lead for denatured hairpin

Call Pittsburgh Public School to ask about lead testing in water fountains (Claire)

Ebony Pugh, media relations (412) 529-3616

Motivation for testing: being proactive in light of recent national events

They installed 3-4 filtered water coolers in each school

Final report should be ready soon

Used 2 recommended tests (couldn't get much more detail about those)

If > 20 ppb detected, shut off the fountain and replace/remove the fixture

Check Plates (Maya)

Growth on PT3-T3-Term and PT7-T3-Term

Liquid cultures (Maya)

PT3-T3-Term, PT7-T3-Term, Terminator, PT7-T3

Prepare for meeting with Dr. Troesken/modeling stuff (Maya)

Thursday, August 25

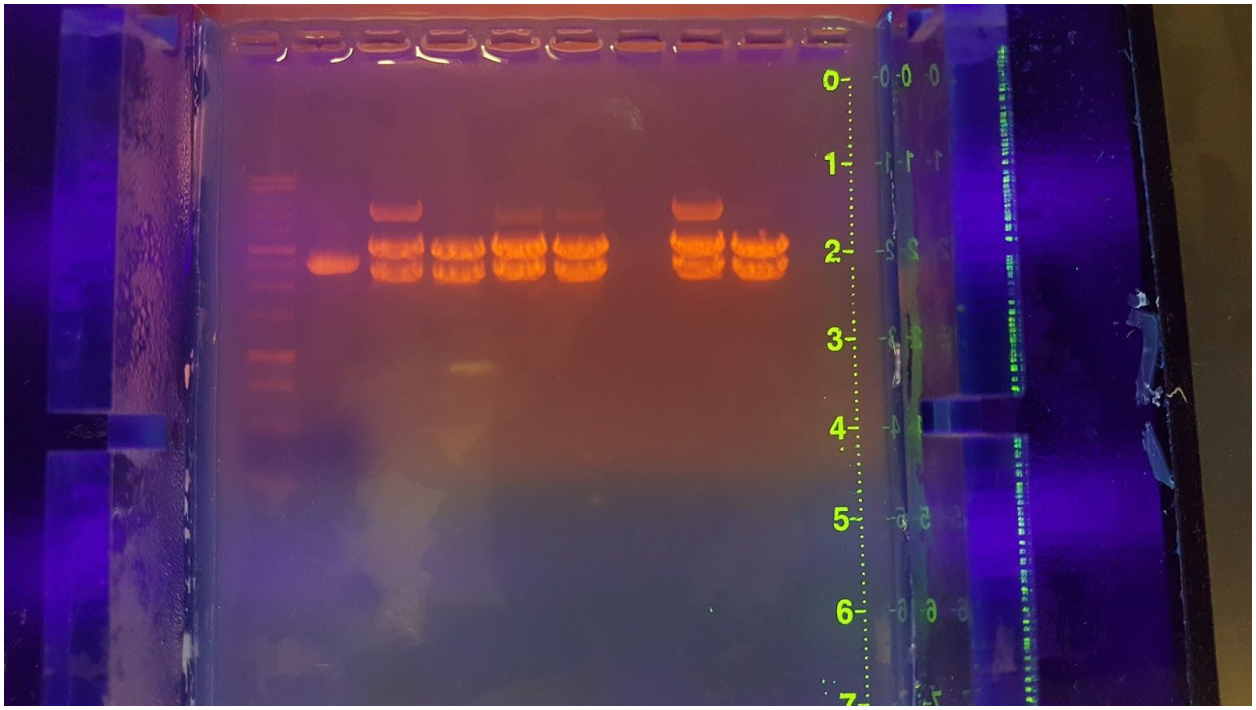
Meeting with Dr. Troesken (Maya)

Miniprepped PT3-T3-Term, PT7-T3-Term, Terminator, PT7-T3 (Maya)

Gel check for ligation (Maya)

PT3-T3-Term and PT7-T3-Term

10 uL reactions, cut with EcoRI and PstI



Lane 1: 1 kb ladder

Lane 2: Terminator control

Lane 3-6: PT3-RBS-T3-Term

Lane 8-9: PT7-RBS-T3-Term

Will two samples of each for sequencing next week as all have double bands indicating good ligation

Friday, August 26

Redo William and Mary in cell-free extract (Claire)

DNA	2 ug
Amino Acids Mix without Methionine	5 uL
Premix without Amino Acids	20 uL
S30 Extract for Circular DNA	15 uL
Water to volume	50 uL

Sample	DNA volume (uL)	Water volume (uL)
A1	7.72	2.28
B1	6.73	3.27
C1	6.58	3.42

D1	7.05	2.96
E1	7.61	2.39
F1	8.74	1.26
G1	7.78	2.22
H1	7.68	2.32
Negative control	0	10

Procedure:

*Keep all tubes on ice

1. Add appropriate amount of DNA and water to 1.5 mL Eppendorf tubes
2. Add AA w/o Met into each tube
3. Add premix to each tube
4. Add extract to each tube and pipette up and down
5. Spin down tubes
6. Aliquot each tube into 2 more tubes of 16.66 uL each; spin
7. Incubate at 37 degC for 1 hour
8. Quench on ice for 5 min; spin down

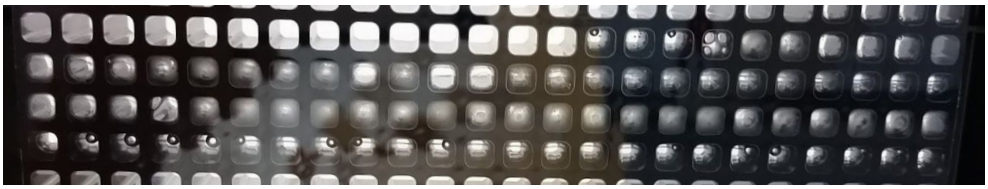
After incubation, add 15 uL of each sample into black 384-well plate

A1 short of 15 uL

Read at 485 excitation, 515 emission, 495 cutoff

Plate:

	1	2	3	4	5	6	7	8	9	10	11	12
E								neg	neg	neg		
H	A	A	A	B	B	B	C	C	C	D	D	D
	13	14	15	16	17	18	19	20	21	22	23	24
H	E	E	E	F	F	F	G	G	G	H	H	H

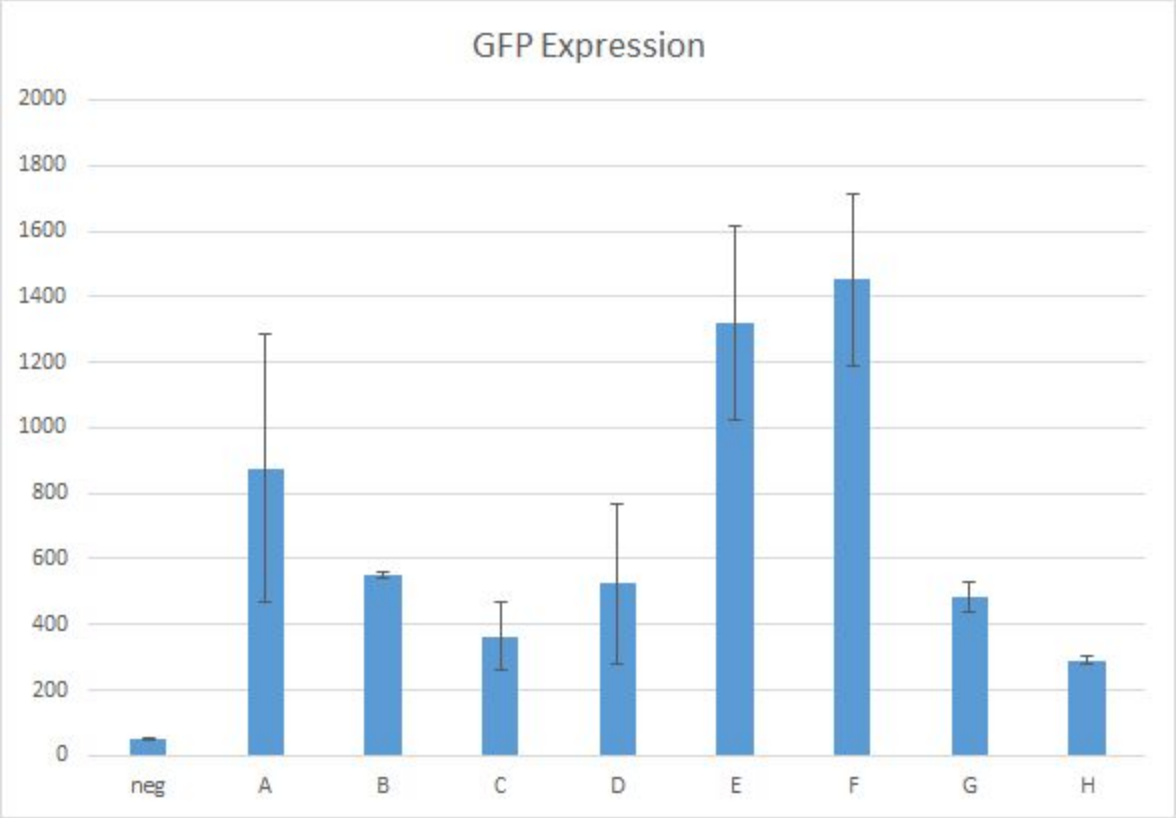


E
F
G
H

Bubbles in most wells

Sample	Replicate 1	Replicate 2	Replicate 3	Avg	St Dev

neg	50.769	44.443	49.099	48.10367	3.278351
A	847.783	486.65	1296.949	877.1273	405.9457
B	537.716	557.127	557.224	550.689	11.23505
C	371.092	466.864	254.967	364.3077	106.1113
D	789.768	308.461	480.132	526.1203	243.9268
E	1378.061	997.871	1585.216	1320.383	297.8903
F	1620.387	1146.405	1585.266	1450.686	264.0995
G	530.228	443.971	477.78	483.993	43.46284
H	305.129	287.992	279.504	290.875	13.0535



A2 is probably an outlier, and A1 would be higher if volume was correct
Relative heights are similar to last time's